

# Research Now

## Supplementing Zinpro Performance Minerals<sup>®</sup> to Young Horses Resulted in a Greater Pro-Inflammatory Response

### Introduction:

Evaluation of the inflammation cascade within the equine joint can be initiated with the presence of lipopolysaccharide (LPS). Cartilage cells produce pro-inflammatory prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in response to the LPS-initiated release of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ). Alterations to cartilage can be detected through examination of synovial fluid. Concentrations of the following peptides indicate joint inflammation status of the horse:

- CPII; collagen synthesis
- C2C; collagen breakdown
- CS-846; aggrecan synthesis (compression bearing constituent of cartilage)

### Experiment Design:

A 56-d study used sixteen weanling Quarter horses (255±22 kg BW), to determine effects of Zn, Mn, Cu, and Co source on intra-articular inflammation and cartilage metabolism. Following an LPS-induced inflammatory challenge on d 42, synovial fluid samples were collected to evaluate markers.

Horses were assigned randomly to 1 of 2 dietary treatments, including iso-levels of supplemental trace minerals formulated to meet or exceed NRC requirements:

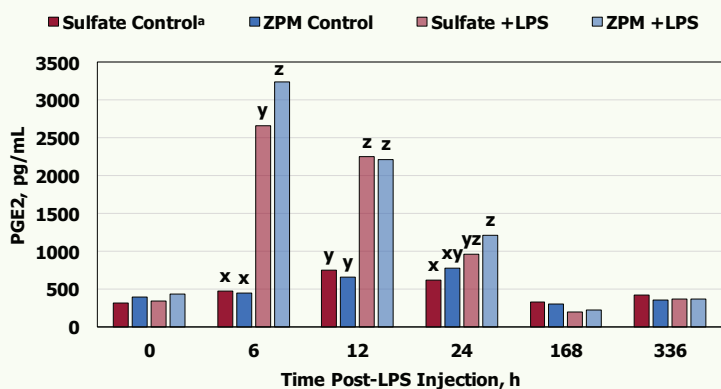
- Sulfate: Zn, Mn, and Cu from sulfates, and Co carbonate
- ZPM: Zn, Mn, and Cu from amino acid complexes and Co glucoheptonate (7 g/hd/d 4-Plex<sup>®</sup>C)

### Results:

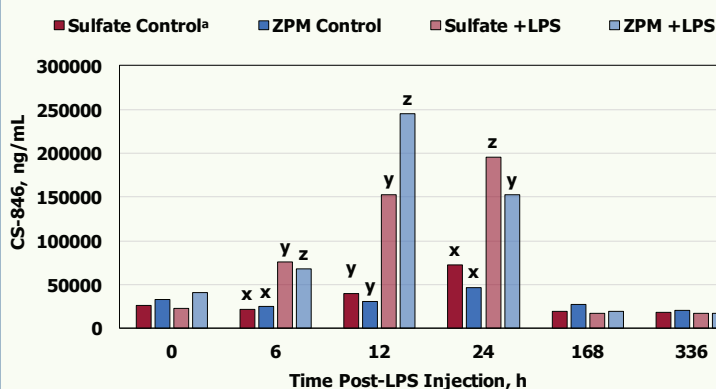
- Intra-articular LPS injection increased synovial PGE<sub>2</sub>, C2C, CPII, and CS846 concentrations across treatments,  $P \leq 0.01$
- In horses fed ZPM, C2C level was increased, compared to those fed Sulfate,  $P < 0.01$  (373.5 vs. 337.4 ng/mL, respectively)
- No overall effect of trace mineral source was observed for PGE<sub>2</sub> ( $P = 0.13$ ); however, horses fed ZPM had greater increase in PGE<sub>2</sub> at 6-h post-LPS injection, compared to CON,  $P < 0.01$  (3239.7 vs. 2664.8 pg/mL, respectively)
- Earlier (12 h) peak for CS-846 level observed for horses fed ZPM,  $P < 0.05$ 
  - CON horses peaked later (24 hr), and with a lower peak CS-486 concentration

These data demonstrate that horses supplemented with Zinpro Performance Minerals<sup>®</sup> produced a more robust pro-inflammatory immune response. This response permitted an increase in collagen degradation, allowing timely joint-repair processes necessary for growing horses.

### Inflammation: PGE<sub>2</sub> Concentration in Synovial Fluid of Knee Joint



### Aggrecan Synthesis: CS-846 Concentration in Synovial Fluid of Knee Joint



<sup>a</sup> Sulfate = Zn, Cu, Mn sulfate and Co carbonate; ZPM = portion of inorganic sources replaced with 4-Plex<sup>®</sup>C; Control = knee injected with saline; +LPS = knee injected with lipopolysaccharide  
<sup>xyz</sup> Within a time point, means lacking a common superscript letter differ:  
 Diet  $P = 0.13$ ; Time  $P < 0.01$ ; Diet  $\times$  Time  $P = 0.25$ ; LPS  $P < 0.01$ ; Diet  $\times$  LPS  $P = 0.17$ ;  
 Time  $\times$  LPS  $P < 0.01$ ; Diet  $\times$  Time  $\times$  LPS  $P = 0.48$

<sup>a</sup> Sulfate = Zn, Cu, Mn sulfate and Co carbonate; ZPM = portion of inorganic sources replaced with 4-Plex<sup>®</sup>C; Control = knee injected with saline; +LPS = knee injected with lipopolysaccharide  
<sup>xyz</sup> Within a time point, means lacking a common superscript letter differ:  
 Diet  $P = 0.61$ ; Time  $P < 0.01$ ; Diet  $\times$  Time  $P = 0.01$ ; LPS  $P < 0.01$ ; Diet  $\times$  LPS  $P = 0.39$ ;  
 Time  $\times$  LPS  $P < 0.01$ ; Diet  $\times$  Time  $\times$  LPS  $P = 0.05$

# Abstract

**Evaluation of dietary trace mineral source on markers of cartilage metabolism in weanling horses challenged with lipopolysaccharide.** A. A. Millican<sup>1</sup>, J. L. Leatherwood<sup>1</sup>, J. A. Coverdale<sup>1</sup>, C. E. Arnold<sup>1</sup>, A. N. Bradbery<sup>1</sup>, K. Fikes<sup>1</sup>, M. S. Goehring<sup>1</sup>, E. C. Dickson, C. M. Latham, C. J. Hartz<sup>1</sup>, C. B Paulk<sup>2</sup>, T. H. Welsh, Jr.<sup>1</sup>, S. H. White<sup>1</sup>, C. K. Larson<sup>3</sup>, E.D. Lamprecht<sup>4</sup>, and T. A. Wickersham<sup>1</sup>, <sup>1</sup>Texas A&M University, College Station, TX, USA, <sup>2</sup>Kansas State University, Manhattan, KS, USA, <sup>3</sup>Zinpro Corporation, Eden Prairie, MN, USA, <sup>4</sup>Cargill Animal Nutrition, Elk River, MN, USA.

Sixteen weanling Quarter horses (255±22 kg BW) were used in a 56-d trial to determine effects of Zn, Cu, Mn, and Co source on intra-articular inflammation and cartilage metabolism following an inflammatory insult. Horses were stratified by days of age (233±20 d), sex (7 fillies, 9 colts), horse source (7 TAMU, 9 Birdsong Farms), and BW. Horses were randomly assigned to dietary treatment formulated with inorganic sources (CON; CuSO<sub>4</sub>, ZnSO<sub>4</sub>, and MnSO<sub>4</sub> and CoCO<sub>3</sub>; n=8) or metal complexes (ZPM; zinc methionine, manganese methionine, copper lysine, cobalt glucoheptonate; n=8). Added trace minerals were at iso-levels between treatments and trace mineral intakes met or exceeded NRC requirements. Horses were offered 1.75% BW treatments (as-fed) and 0.75% BW coastal bermudagrass (*Cynodon dactylon*; as-fed) hay. Total diet was split into two equal feedings daily at 0600 and 1600 h. Daily DM intakes did not differ between treatments ( $P > 0.05$ ). On d 42, an intra-articular lipopolysaccharide (LPS) challenge was conducted. Carpal joints were randomly assigned to receive intra-articular injections of 0.5 ng LPS derived from *Escherichia coli* 055:B5 or sterile lactated Ringer's solution as a contralateral control. Synovial fluid was collected at pre-injection h 0 and 6, 12, 24, 168, and 336 h post-injection. Samples were analyzed for prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), carboxypeptide of type II collagen (CPII), collagenase cleavage neopeptide (C2C) and aggrecan chondroitin sulfate 846 epitope (CS846) using commercial ELISA kits. Data were analyzed using the MIXED procedure of SAS. Intra-articular LPS increased ( $P \leq 0.01$ ) synovial PGE<sub>2</sub>, C2C, CPII, and CS846 concentrations regardless of treatment. Synovial C2C was greater ( $P < 0.01$ ) in ZPM horses (373.5±9.3 ng/mL) compared to CON (337.4±9.3 ng/mL). In contrast, CPII concentration remained unaffected by treatment. Despite differences in degradation, the ratio of anabolic to catabolic processes (CPII:C2C) was unaffected by diet ( $P = 0.57$ ). No effect of diet was observed for PGE<sub>2</sub> ( $P = 0.13$ ); however, ZPM horses had a greater increase in concentration of PGE<sub>2</sub> in the LPS-injected joint at 6 h compared to CON ( $P < 0.01$ ; 3239.7±116.2 and 2664.8±116.2 pg/mL, respectively). A diet × time × LPS interaction was observed for CS846. Concentrations for ZPM horses peaked at 12 h while CON horses peaked with a lower concentration at 24 h ( $P < 0.05$ ). Compared to inorganic mineral sources, data suggest that sufficient intake of a complexed trace mineral source may support a more robust proinflammatory immune response to an LPS challenge resulting in increased collagen degradation and a more rapid rise in aggrecan reparative processes in young growing horses.

**Key words:** cartilage, equine, lipopolysaccharide, trace mineral

2018 American Society of Animal Science - Southern Section Annual Meeting, February 3-6, 2018, Ft. Worth, TX, USA